

PATENT SPECIFICATION

(11) 1356 449

1356 449

- (21) Application No. 34644/71 (22) Filed 23 July 1971
 (31) Convention Application No. 128818 (32) Filed 30 Dec. 1970
 (31) Convention Application No. 33119 (32) Filed 17 May 1971
 (31) Convention Application No. 47835 (32) Filed 30 June 1971 in
 (33) Japan (JA)
 (44) Complete Specification published 12 June 1974
 (51) International Classification A61K 27/14
 (52) Index at acceptance A5B 779



(54) A PROCESS FOR EXTRACTING WATER SOLUBLE COMPONENTS OF THE HYPHAE OF EDIBLE FUNGI

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The present invention relates to the extraction of water soluble components of the hyphae of edible fungi. More specifically, the invention is directed to a new and advanced process for the extraction of water soluble components having therapeutic effects of the hyphae of edible fungi that include, perhaps most importantly, "Shiitake" which is a popular Japanese term for *Lentinus edodes* of the class Basidiomycetes prevailing in East Asia.

Whilst so-called "mushrooms" have been prized simply as food delicacies in most Western cultures, it has long been recognized in the countries of the Orient that some species of edible fungi contain substances having a curative effect on a wide variety of diseases and disorders of mankind. The ancient Chinese, in particular, were perhaps most aware of this fact, as evidenced by many writings they left which attest to the therapeutic value of Shiitake and other edible fungi.

For all their acknowledged virtues, however, the desired useful substances hereinafter to be described in detail have hitherto been extracted only by an age-old process of decoction, wherein the fruiting bodies of a desired edible fungus are boiled for a prescribed length of time. The liquid extract thus obtained is not as effective as it should be. Moreover, the fruiting bodies of the edible fungi are not available in large quantities, or only at high cost so that extraction of the useful ingredients thereof is not currently practised at any industrial level.

Accordingly, the present invention provides a process for extracting water soluble components of the hyphae of an edible fungus which comprises cultivating the hyphae of the edible fungus in a prepared culture medium comprising one or more solid nutrients for the edible fungus, reducing the hyphae and the culture medium to fine particles approximately at the time when the said hyphae begin fruiting, forming an aqueous suspension of said fine particles, and filtering the suspension to remove undesirable matter therefrom and to provide an aqueous solution of an extract of the edible fungus.

The hyphae culture may be subjected to a rapid reduction in temperature and/or a change in humidity such as to accelerate the growth of the hyphae.

The culture may be rapidly reduced in temperature from room temperature to a temperature of from 4°C to 8°C. After being maintained at said reduced temperature for a period of time, the culture may be immersed in water having a temperature of substantially 5°C.

Whilst the process of the present invention is applicable to a variety of edible fungi, the aforementioned Shiitake is perhaps most appropriate in view of the ingredients of the extract obtained therefrom, as hereinafter described in detail. As an added advantage Shiitake can be artificially cultivated with comparative ease.

It is known that the cell membranes of the hyphae Shiitake and some other edible fungi are composed primarily of β - 1 - 3 glucane and chitin, and that the cell membranes are dissolved by β 1 - 3 glucanase and citanase which are enzymes produced in the hyphal cells through the metabolic processes thereof. Further experiments made by the present applicants have proved that the concentration of therapeutically effective substances, including the aforesaid enzymes, in the hyphal cells of the edible fungi is most pronounced at the stage of transformation into fruiting bodies from their secondary hyphae.

Although the exact reasons for this are as yet unascertained, it is inferred from the available evidence that this may be because the hyphal cells are most metabolically active when at said stage.

Hence the present invention proposes to extract the useful substances from the hyphae of edible fungi and not from their fruiting bodies. This is dually advantageous because the extracts obtained from the former are more therapeutically effective than those from the latter and because the former is considerably less costly than the latter.

The desired hyphae for the process of this invention may be cultured in any suitable culture media such as a medium comprising logs, agglomerated sawdust or bagasse. Although the hyphae can be cultivated in a liquid medium, it has been experimentally confirmed that hyphae grown in a solid media are more suitable for processing into extracts having desired effects. The various culture media employed in the practice of the process of the invention may be prepared substantially just as they have been in the past. However, in case of sawdust culture, the ratio by weight of sawdust content to that of rice bran is preferably substantially 9 to 1 for the best results, as compared to the 7:3 ratio used in the prior sawdust culture.

Although a culture medium inoculated with a seed culture of a desired edible fungus may be kept in usual conditions, experiments have proved that the hyphae grown therefrom should be subjected to rapid change in temperature and/or humidity at a suitable moment so as to stimulate and accelerate the growth or metabolic processes thereof. Preferable environmental changes to be given to the hyphae are detailed later in descriptions of some Examples of the process of this invention.

Inasmuch as the hyphae just going to form fruiting bodies contain the maximal concentration of useful substances as previously mentioned, it is essential, or at least highly desirable, that they should be processed at that time. This time is ascertainable by some obvious signs. First, a culture medium containing sufficiently grown hyphae will turn white at the boundaries immediately before formation of fruiting bodies. Second, as far as Shiitake and some allied species are concerned, a sort of swelling phenomenon will be noted on their hyphal mats when the hyphae have sufficiently grown in their culture media.

The appropriately grown hyphae of a selected edible fungus together with culture medium may be pulverized into as fine particles as feasible by suitable means. The pulverized materials may then be introduced into distilled and sterilized water having a pH of about 5.0. The suspension thus prepared may be charged into a sealed vessel,

heated to a temperature at which the production of enzymes in the hyphae is accelerated, and filtered to provide a desired extract. For the best results the suspension should be heated in two successive steps, first to a temperature of from 30°C to 35°C for substantially one hour and then at a temperature of from 45°C to 55°C for one to two hours. The first heat treatment is to accelerate the production of β 1 - 3 glucanase, chitinase and other enzymes of the hyphae, whereas the second heat treatment is to stimulate the activities of those enzymes.

The aqueous extract thus obtained may be admixed with a suitable amount of ammonium sulphate so as to precipitate the useful substances contained in the extract, the precipitate collected, the collected precipitate dissolved in distilled and sterilized water, and the resulting solution dialyzed by means of a semipermeable membrane to remove any remaining ammonium sulphate. The semipermeable membrane should be such that it permits the passage of particles having a molecular weight of not more than substantially 1,000. Further, the amount of ammonium sulphate added to the filtrate has to be rather carefully controlled. It has been experimentally proved that the best results are obtained when about 584 grams of ammonium sulphate is mixed with about 1,000 milli-litres of the filtrate.

An analysis of the extract obtained from Shiitake hyphae by the above described process of the invention has revealed the following ingredients: 40 enzymes including β - 1 - 3 glucanase and chitinase, 18 amino acids, vitamins B₂ and B₁₂, ergosterin, polysaccharides and polypeptides. It is quite likely, however, that the extracts include other useful substances yet unknown.

Described hereinbelow are only some of the results of the use of an extract prepared from Shiitake hyphae in accordance with the teachings of the present invention:

1. A 42-year-old female, who had been suffering for years from high blood pressure of maximum 210 mmHg and minimum 160 mmHg, took 100 ml of the extract three times a day before meals. In four days the blood pressure was lowered to maximum 160 mmHg and minimum 100 mmHg and thereafter has stabilized in that range.

2. A 40-year-old male, whose tonsils looked white due to tonsillitis, took 200 ml of the extract and completely recovered 24 hours later.

3. A 36-year-old male, who had a fever of about 39°C with a cold, took 200 ml of the extract three times a day before meals. In 24 hours the fever subsided.

4. A 41-year-old male, seriously affected by vesicular eczema, had 50 ml of the extract applied to the affected parts four times a day.

In two days the eczema disappeared and has not recurred.

5. A 42-year-old male, with a serious case of stomach cancer, took 50 ml of the extract three times a day for a period of 30 days, at the end of which signs of improvement were noted (no well-founded explanation for this is yet available).

6. After being heated at a temperature ranging from 30°C to 35°C and then dried to a 20% by weight moisture content, a small amount of culture containing suitably grown Shiitake hyphae was added to a feed-stuff. The cell walls of Eumycetes that had been grown in the feedstuff were dissolved by the activities of β - 1 - 3 glucanase and chitinase introduced by the added culture, thereby resulting in the protection of the animals against possible infectious diseases. It also appears that the aforesaid enzymes, with their activities accelerated by the intestinal heat of the animals, dissolved the cell bodies of the Eumycetes into amino acids that play an important role in the metabolism and growth of the animal tissues.

7. Experiments have proved that the activities of β - 1 - 3 glucanase and chitinase in the culture containing suitably grown Shiitake hyphae, which has been treated substantially as aforesaid, destroy various undesirable fungi growing in soil when the culture is mixed therewith. The enzymes are also known to stimulate the growth of useful bacteria and microorganisms contained in such soil.

The present invention is hereinafter described more specifically according to some Examples thereof, which are meant only to illustrate and not to impose limitations on the invention.

EXAMPLE I

One kilogram of a culture medium containing bagasse and distilled water with a pH of about 5.0 in such a ratio by weight that its moisture content ranges from 60% by weight was placed in a suitable vessel and was autoclaved at 121°C for 20 minutes for sterilization purposes. The sterilized nutrient medium was inoculated with a seed culture of Shiitake and was then allowed to stand for 60 days in an air-conditioned chamber having a substantially constant temperature of about 25°C. For the desired temperature change the culture was then moved into a chamber ranging from 4°C to 8°C in temperature. After being allowed to stand for 72 hours in the low temperature chamber, the culture together with its vessel was immersed in water at a temperature of about 5°C for 24 hours. As the aforementioned swelling phenomenon was observed on the Shiitake hyphae now covering the entire surface of the medium, the hyphae together with part of the medium was pulverized into fine particles.

Three hundred grams of the particles thus formed was added to about 1,000 millilitres of distilled and sterilized water having a pH of about 5.0. The suspension thus obtained was first heated at about 30°C for 1 hour and then at about 50°C for 2 hours in a sealed vessel. Thereafter the suspension was cloth-filtered under pressure and then dialyzed by means of a membrane filter with a pore size of about 0.45 μ to obtain about 600 millilitres of an aqueous extract containing the useful substances.

EXAMPLE II

The process of Example I was faithfully repeated only with the described culture medium replaced with one containing the sawdust of a broad-leaved tree and rice bran in the ratio of about 9 to 1 by weight and having its moisture content regulated to from 60 to 65% by weight by the addition of distilled water with a pH of about 5.0. About 620 millilitres of an aqueous extract was similarly obtained.

EXAMPLE III

A seed culture of Shiitake was inoculated on a log of Japanese oak (*Quercus glandulifera*) and cultivated in the ordinary way. Approximately at the moment when the propagated hyphae started fruiting, they were pulverized together with the log. One kilogram of the particles thus formed was added with 1,000 millilitres of distilled and sterilized water having a pH of about 5.0. Thereafter the suspension was processed substantially as described in Example I to provide about 700 millilitres of an aqueous extract.

EXAMPLE IV

One thousand millilitres of an aqueous extract of Shiitake obtained according to any one of the foregoing Examples I to III was admixed with about 584 grams of ammonium sulphate. The solution was allowed to stand for 24 hours at room temperature thereby to cause precipitation of the useful substances that were contained in the aqueous extract. The precipitate, collected by filtration, was then thrice rinsed with a near saturated solution of ammonium sulphate, with intervening steps of filtration. Ten grams of the precipitate (in the wet state) so treated was charged into about 100 millilitres of distilled and sterilized water having a pH of about 5.0. The solution was dialysed by means of a semipermeable membrane to obtain about 35 millilitres of ammonium-sulphate-free solution that contained the useful substances. This sample, after being suitably admixed with distilled and sterilized water and again dialysed for complete sterilization, was administered to the several patients described earlier in this specification.

WHAT WE CLAIM IS:—

1. A process for extracting water soluble components of the hyphae of an edible fungus which comprises cultivating the hyphae of the edible fungus in a prepared culture medium comprising one or more solid nutrients for the edible fungus, reducing the hyphae and the culture medium to fine particles approximately at the time when the said hyphae begin fruiting, forming an aqueous suspension of said fine particles, and filtering the suspension to remove undesirable matter therefrom and to provide an aqueous solution of an extract of the edible fungus.
2. A process according to claim 1, in which the hyphae culture is subjected to a rapid reduction in temperature and/or a change in humidity such as to accelerate the growth of the hyphae.
3. A process according to claim 2, in which the said culture is rapidly reduced in temperature to a temperature of from 4°C to 8°C.
4. A process according to claim 3, wherein, after being maintained at said reduced temperature for a period of time, the culture is immersed in water having a temperature of substantially 5°C.
5. A process according to any preceding claim, in which the said suspension is heated in a sealed vessel, before being filtered, to a temperature at which the production of enzymes in the hyphae is accelerated.
6. A process according to claim 5, in which the said suspension is heated first to a temperature of from 30°C to 35°C for substantially one hour and then to a temperature of from 45°C to 55°C for one to two hours.

7. A process according to any preceding claim, and further comprising the steps of admixing the obtained aqueous extract with ammonium sulphate so as to precipitate the useful substances contained in the extract, collecting the precipitate, dissolving the precipitate in water, and dialyzing the resulting solution to remove any remaining ammonium sulphate.
8. A process as claimed in any one of the preceding claims, in which the said culture medium comprises bagasse, sawdust or log.
9. A process according to claim 8, in which the culture medium comprises sawdust and rice bran, the ratio by weight of sawdust content to that of rice bran in the prepared culture medium being substantially 9 to 1.
10. A process according to any one of the preceding claims, in which the said edible fungus is Shiitake.
11. A process for extracting water soluble components of the hyphae of an edible fungus according to claim 1 and substantially as herein disclosed.
12. A process for extracting water soluble components of the hyphae of an edible fungus, substantially according to any of the specific Examples herein.
13. Water soluble components of the hyphae of an edible fungus when extracted by the process according to any one of the preceding claims.

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Printed for Her Majesty's Stationery Office, by the Courier Press, Leamington Spa, 1974.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.